Siderophore Receptor and Porin Protein Technology for Control of *Salmonella* and *Escherichia coli* O157:H7 in Cattle

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Abstract

Siderophore receptor and porin protein vaccine technology is a new tool to control clinical and subclinical bacterial disease in cattle through protection of exposed animals and decreased shedding in animals with enteric bacterial colonization. Data in this review have shown that the immune system of animals normally doesn't recognize SRP proteins. However, if primed the immune system will continue to increase the titer against these proteins without re-vaccination. These novel subunit *Escherichia coli* and salmonella vaccines have shown to decrease mortality and condemnation while increasing the performance in fed turkeys. The salmonella and *E. coli* O157:H7 vaccines have shown to prevent mortality and decrease shedding of both salmonella and *E. coli* O157:H7 in mice and cattle. The decrease in shedding and protection from subsequent disease leads to increased production, as illustrated by the results in dairy cow studies. After effectively demonstrating control of salmonella and *E. coli*, it is exciting to think of the potential that SRP vaccine technology has for control of numerous bacterial disease complexes in cattle such as bovine respiratory disease complex (BRDC), liver abscesses, footrot and pink eye.

Introduction

A number of important disease complexes of cattle, such as salmonellosis, are either caused or exacerbated by bacteria. There also are bacteria such as *E. coli* O157:H7 that colonize in the gut of cattle and don't cause disease. Although there is no adverse outcome in cattle, *E. coli* O157:H7 has been shown to cause disease in humans. Antibiotic usage for such pathogens is sometimes warranted for treatment of disease. However, future control of bacterial pathogens that cause disease in cattle, or disease in humans because of shedding from cattle, will not involve low levels of antimicrobials. Therefore unique, efficacious technology must be delivered to decrease bacterial organisms in cattle without usage of antimicrobials.

While many vaccines are commercially available for immunization against individual species and serotypes of bacteria, few, if any, provide adequate cross-protection or stimulate broad-based immunity against multiple serotypes or species. One essential factor required for a bacterium to induce clinical disease is the ability to proliferate successfully in the host. Iron is an essential nutrient for growth of gram-negative bacteria in the host. However, it is virtually unavailable in host tissue because the majority of iron is found intracellularly, or a minor amount may be complexed with high-affinity iron binding proteins extracellularly. To circumvent low iron environments, pathogenic bacteria have evolved a high-affinity iron transport system produced under low iron-concentrations, which consists of siderophores and iron-regulated outer membrane proteins (IROMP), and/or siderophore receptor proteins, which are receptors for siderophores found on the outer membrane of the bacterial cell. Siderophores are made and secreted under low iron conditions by gram-negative bacteria to scavenge or steal extracellular iron away from the host (Figure 1).

A novel vaccine technology developed by Epitopix (Willmar, MN) exploits a pathogenic bacteria's intrinsic need for iron. Siderophore receptor and porin proteins (SRPs) technology uses purified siderophore receptor and porin proteins from specific bacterial species (salmonella, *E. coli,* etc...) as an immunogen. SRP-vaccinated animals recognize the bacterial SRP proteins as foreign and mount an appropriate immune response. The culmination of the immune response is the development of memory cells that upon challenge will induce the production of antibodies to the bacterial SRPs. The anti-SRP antibodies bind the siderophore receptor proteins present on the bacteria's outer membrane and block the transport of siderophores complexed with iron. Ultimately, this mechanism of iron transport blockage leads to death of the corresponding bacterial species used for the development of the SRP vaccine (salmonella SRP vaccine, *E. coli* O157:H7 vaccine, mannheimia SRP vaccine, etc…).

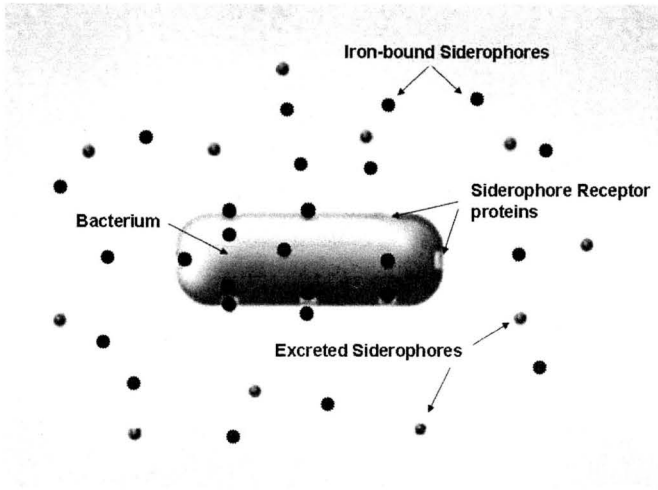

Figure 1. In low-iron environments, pathogenic bacteria produce siderophores, which are capable of scavenging or stealing iron from the host. The iron-bound siderophore is recognized by siderophore receptor proteins present on the bacteria's cell surface. Siderophore receptor proteins facilitate transfer of iron into the bacteria.

SRP Vaccine Animal Studies

Salmonella and *E. coli* can affect health and performance of many food-producing animals. A siderophore receptor and porin protein vaccine for *S. newport* for use in cattle is currently marketed by Agrilabs, Inc (St. Joseph, MO). The fundamental and field research that led to the approval of this vaccine was conducted on turkeys, mice and cattle.

Poultry

Willmar Poultry Company, parent company of Epitopix, monitors approximately 50 commercial turkey breeder flocks for subclinical salmonella shedding by taking 500 individual cloacal swabs per flock. If one individual sample is positive, the flock is designated positive. From 1986 to 1996, the flocks were consistently > 90% positive. This, despite the following prevention steps: salmonella autogenous bacterins, shower in/shower out facilities, professional rodent control, wild bird exclusion, semi-annual formaldehyde barn washes, formaldehyde in feed, no animal protein in feeds, extruded feed, separate feed trucks and service trucks for breeder operation, use of direct feed microbial and separate breeder hatching facility.

In 1996, 94% of the Willmar Poultry breeder flocks were positive for salmonella. The veterinarians then implemented SRP technology salmonella vaccines in the breeder flocks in 1996, taking 18 months for the complete operation to be vaccinated. Vaccination along with continued biosecurity procedures reduced salmonella prevalence in the breeder flocks to 9% by 2001. Although these studies were not controlled, performance and health of the flocks greatly improved.

Use of SRP technology to combat *E. coli* infections, both clinical and subclinical, has also been established in turkeys. Turkeys vaccinated once with an *E. coli* SRP vaccine had good titer responses to SRP proteins. Interestingly, the titer responses in vaccinates continued to rise for a six-to-10-week period without revaccination. The non-vaccinate animals did not show titer responses to SRP proteins. This illustrates that the immune system of animals does not normally recognize SRP proteins without priming.

Does an SRP vaccine affect performance? Energy utilized to drive an immune response can be partitioned away from energy utilized for growth. Understanding that one dose of SRP vaccine leads to ramping titers could be of concern when looking at performance. The results from vaccinating turkeys with an *E. coli* SRP vaccine (n = 1,130,862 birds; 362,169 and 768,693 vaccinates and controls, respectively) are shown in Table 1. Turkey vaccinates that received an *E. coli* SRP vaccine had decreased mortality (38.5%, $P < 0.01$), decreased condemnation (31%, $P < 0.01$) and improved weight at processing (9.3%, $P < 0.01$) relative to non-vaccinates.

Mice

Mouse models are excellent ways of testing *S. newport* and *E. coli* vaccines. In an *S. newport* challenge study, mice received different concentrations of the same *S. newport* vaccine: 1) stock solution 50 ug/0.1 ml, 2) 1:10 (volume diluent:volume vaccine, respectively), 3) 1:100, 4) 1:1000 and a placebo control. Adjuvant concentrations were similar across all treatments. Mice were vaccinated intraperitoneally on day 0 and day 14 with 0.1 ml of the assigned vaccine treatment. On d 28 (14 days after second vaccination), mice were intraperitoneally challenged with *S. newport*.

Table 1. Effects of an *E. coli* SRP vaccine on the health and performance of turkeys.

	Vaccinated (n = 362,169)	Control (n = 768,693)
Mortality % (± sd)	7.48 ± 1.08*	12.16 ± 6.05*
Condemnation % (± sd)	1.07 ± 0.17*	1.55 ± 0.61*
Weight at processing (lb)	15.21 ± 0.69*	13.91 ± 0.67*
Age processed (days)	94	96

The results show a strong indication that: 1) SRP *S. newport* vaccine protected mice against an *S. newport* challenge, and 2) dilution of the SRP vaccine had a significant effect on efficacy (Table 2). All 25 of the non-vaccinate controls died within 14 days post-challenge. Conversely, the groups given stock solution or the 1:10 dilution only had 4% (1 out of 25 mice) and 8% (2 out of 25 mice) mortality, respectively. Dilution of the stock solution to 1:100 and 1:1000 was not as protective as the stock solution or 1:10 dilution against salmonellosis in mice.

Salmonella and *E. coli* can be serious environmental pathogens. Reduced shedding of these pathogens could be valuable in decreasing exposure to other animals. Two studies were completed to examine the effects of SRP vaccines for salmonella and *E. coli* shedding by mice.

The first study used 20 (two treatments, 10 mice per treatment) mice to observe the effects of SRP technology on the fecal shedding of *S. newport*. The mice were administered either a placebo control or a vaccine treatment (days 0, 14 and 28 with an *S. newport* SRP vaccine). Seven days after the last vaccination all mice were orally challenged with *S. newport*. Fecal shedding of salmonella was similar between vaccinates and controls at the time of challenge (Figure 2). However, at 12, 24, 36 and 48 hours post-challenge the vaccinates had significantly lower fecal shedding than the placebo group.

In the same study, mortality was 30% for vaccinates and 70% for placebo controls. Interestingly, mortality was highly correlated with the amount of shedding. Furthermore, vaccinate mortality took place within the first 12 hours post-challenge. Mortality in the placebo group occurred continually throughout the post-challenge period. However, as fecal shedding declined so did mortality.

A similar study was completed to examine the effects of an SRP technology vaccine for *E. coli* O157:H7 on fecal shedding in mice (Figure 3). A nalidixic acid-resistant *E. coli* O157:H7 isolate was used for the challenge. Again, 20 mice (two treatments, 10 mice per

Table 2. Mortality of vaccinated and non-vaccinated mice following challenge with *Salmonella newport*.

Groups	No. mice	No. dead	Mortality (%)
Group-1 (non-diluted)	25	1/25	4.0
Group-2 (1:10)	25	2/25	8.0
Group-3 (1:100)	25	12/25	48.0
Group-4 (1:1000)	25	15/25	60.0
Group-5 (non-vaccinated/challenged)	25	25/25	100.0

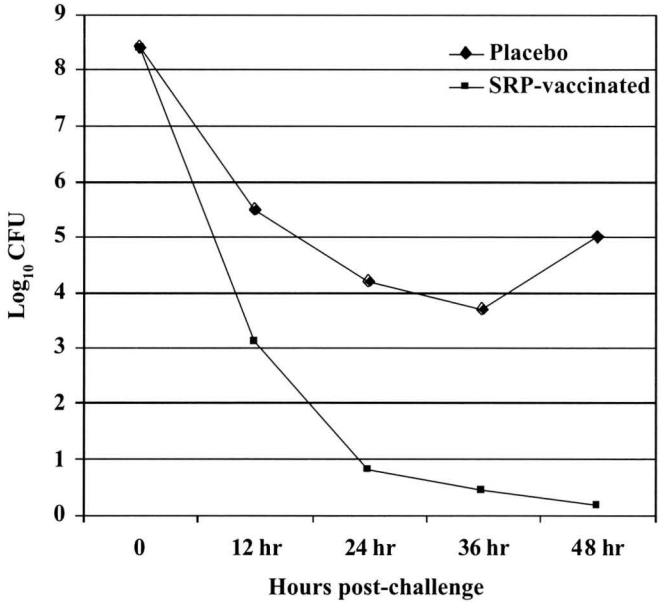

Figure 2. The difference in fecal shedding between vaccinated and non-vaccinated mice after oral challenge with *Salmonella newport*.

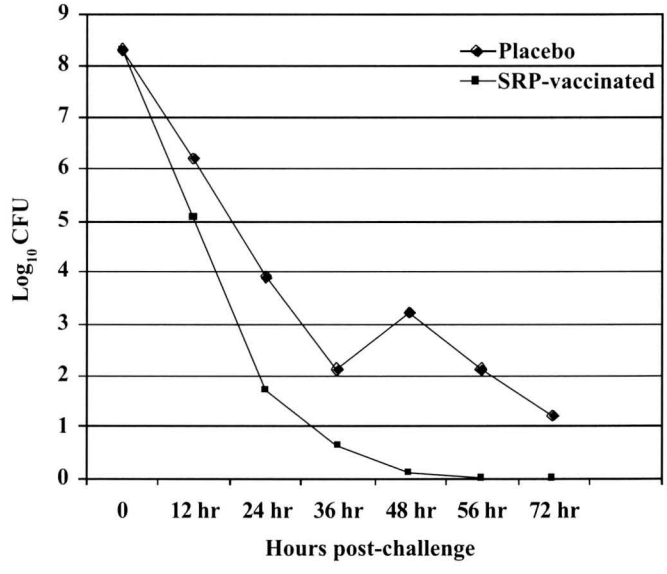

Figure 3. The difference in fecal shedding between SRP-vaccinated and non-vaccinated mice after oral challenge with *E. coli* 0157:H7.

treatment) were used for this challenge study. The treatments were: 1) placebo control and 2) vaccinated on d 0, 14, and 28 with an SRP *E. coli* O157 vaccine. All mice were orally challenged at seven days after the third vaccination with *E. coli* O157. Fecal samples were collected at 0, 12, 24, 36, 48 and 72 hours post-challenge.

The SRP vaccination was very effective in controlling the shedding of *E. coli* O157:H7 in mice (Figure 3). At 24 hours post-challenge, vaccinates were shedding half as much *E. coli* O157:H7 compared to the placebo controls. Vaccinates essentially stopped shedding *E. coli* O157:H7 by 48 hours post-challenge. These data indicate that vaccination with SRP technology can significantly decrease the amount of shedding of *E. coli* O157:H7 in mice following an oral challenge.

Cattle

SRP studies in mice have paved the way for development of SRP vaccines that seek to eliminate salmonella and *E. coli* in cattle. In a perfect world, all licensed vaccines would undergo a rigorous challenge study in the field, away from sterile isolation laboratories. Unfortunately, there are many products marketed today that show statistically significant results in a laboratory or an isolation facility but have little or no effect on an operation's bottom line. Again, SRP technology products were developed by a production company to fix production and health problems. Many times in our industry, problems are developed because we have products to sell/fix them.

An *E. coli* O157:H7 SRP vaccine challenge study used 12 Holstein steer calves to observe its effects on fecal shedding after oral challenge with *E. coli* O157:H7. The treatments were: 1) control (no vaccination), 2) vaccinated on days 0, 18, and 36 with SRP *E. coli* O157:H7 antigen with an aluminum hydroxide adjuvant or 3) vaccinated on d 0, 18, and 36 with SRP *E. coli* O157:H7 antigen with an Emulsigen adjuvant. Calves were transported to the isolation facility four days after the last vaccination. Once calves were at the isolation facility for four days, they were challenged orally with a nalidixic acid-resistant strain of *E. coli* O157:H7. Fecal samples were taken from each steer at 12, 24, 48, 72, 96, 120, 144 and 168 hours post-challenge.

Data from the study indicated that SRP *E. coli* O157:H7-vaccinated cattle with an oil adjuvant had decreased *E. coli* O157:H7 shedding compared to non-vaccinate controls and those vaccinated with the aluminum hydroxide adjuvant. Furthermore, by 48 hours post-challenge, there was a 100% decrease in shedding in the oil-adjuvanted SRP *E. coli* O157:H7 vaccinates when compared to the non-vaccinate controls. This response remained constant through the 168-hour sampling period. These data indicate that in a challenge situation SRP *E. coli* O157:H7 vaccination can decrease the fecal shedding of *E. coli* O157:H7 in cattle.

Control of salmonella in dairy operations is difficult even with the best biosecurity procedures. Morbidity and mortality due to this disease complex can be costly to producers. Like many diseases, clinical manifestation is not always achieved and subclinical problems can decrease profitability. An SRP salmonella field study was conducted in a 500-cow expansion dairy herd. This herd was not showing any outward signs of clinical salmonellosis. Two groups of cows were selected for this study: 1) fresh cows (30 to 90 days post-partum) and 2) dry cows (21 to 60 days prepartum). Cows in each group were randomly assigned to a control group or to a salmonella SRP vaccine group.

Fresh cows received two vaccinations 28 days apart. The dry cows were vaccinated at dry-off and again two to three weeks prior to parturition. Results from both the fresh cow (Figures 4 and 5) and the dry cow

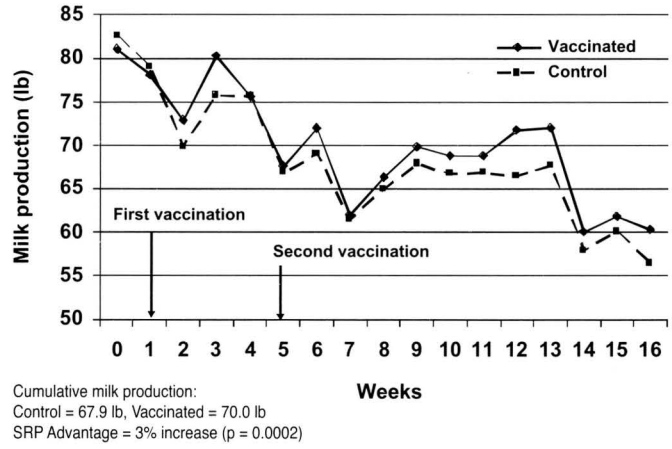

Figure 4. Average weekly milk production comparing SRP-vaccinated and non-vaccinated fresh cows.

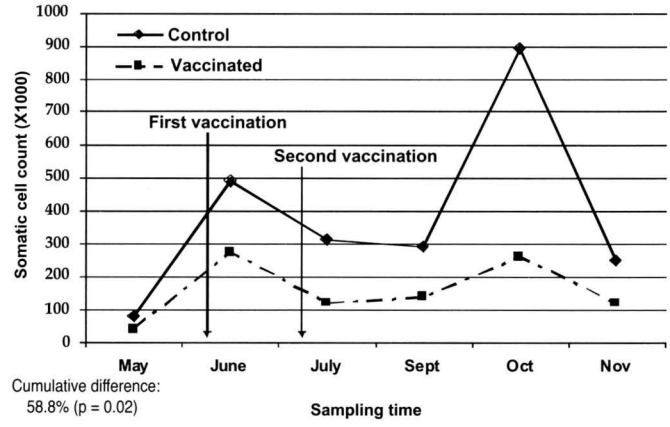

Figure 5. Monthly average somatic cell count comparing SRP-vaccinated and non-vaccinated fresh cows.

studies (Figure 6) were very positive. Average weekly milk production was increased in the fresh cows vaccinated with the salmonella SRP vaccine by 3% (P = 0.0002) relative to the unvaccinated controls (Figure 4). Fresh cows vaccinated with salmonella SRP vaccines also had lower cumulative somatic cell (30% decrease, P = 0.036) relative to the non-vaccinated controls (Figure 5).

Vaccinating dry dairy cows before they come back into production with a salmonella SRP vaccine was positive on milking performance. Dry dairy cows vaccinated with a salmonella SRP vaccine twice before parturition had significantly higher milk yields during the first 30 days of production (13.1 lb; 5.95 kg; $P < 0.0001$) than non-vaccinated cows (Figure 6). Vaccinated cows also had a decrease in somatic cell count (13%, $P < 0.01$) relative to the non-vaccinate controls. Both studies indicate the potential for salmonella SRP vaccination in dairy production. Milk yield responses to SRP vaccination were greater in the study when cows were vaccinated prepartum relative to postpartum. However, somatic cell count responses to SRP vaccination were better in the study in which cows were vaccinated postpartum. More studies need to be conducted to understand the optimum timing and doses of salmonella SRP vaccination for minimizing health problems while maximizing performance of dairy cows.

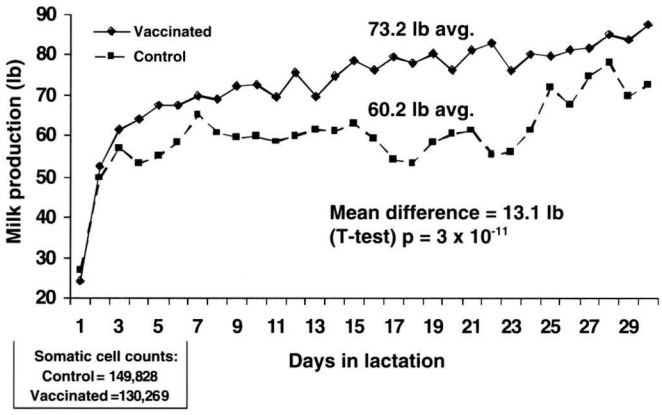

Figure 6. Average daily milk production comparing SRP-vaccinated and non-vaccinated dry cows in the first 30 days of production.

Conclusions

Siderophore receptor and porin protein vaccine technology is a new way to control clinical and subclinical bacterial disease in cattle through protection of exposed animals and decreased shedding in animals with enteric bacterial colonization. Data in this review have shown that the immune system of animals normally doesn't recognize SRP proteins, but if primed the immune system will continue to increase the titer against these proteins without re-vaccination. These novel subunit *E. coli* and salmonella vaccines have shown to decrease mortality and condemnation while increasing performance in fed turkeys. The salmonella and *E. coli* O157:H7 vaccines have shown to prevent mortality and decrease shedding of both salmonella and *E. coli* O157:H7 in mice and cattle. The decrease in shedding and protection from subsequent disease leads to increased production as illustrated by the results in dairy cow studies. After effectively demonstrating control of salmonella and *E. coli*, it is exciting to think of the potential that SRP vaccine technology has for control of numerous bacterial disease complexes in cattle such as BRDC, liver abscesses, footrot and pink eye.

Aside from improved health and performance, we now have to focus on the safety of the products we produce. The reduction of food-borne pathogens in beef, specifically *E. coli* O157:H7, has been the focus of many research groups around the world. The results presented within this review article from the mice and cattle challenge studies are quite promising, and indicate a potential for the SRP *E. coli* O157:H7 vaccines to control shedding in cattle. SRP vaccines are here to stay, and it will be exciting to see where their diverse application will take us.